Ⅳ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表（雑誌）

発表者氏名	論文タイトル名	発表雑誌	巻号	ページ	出版年
Nomura Y, **Nomura T**, Sakai K, Sasaki K, Mizuno O, Hata H, Aoyagi S, Abe R, Itaya Y, **Akiyama M**, **Shimizu H**	A mutation in the genes encoding γ-secretase underlies familial but not non-familial hidradenitis suppurativa.	**Br J Dermatol**			投稿中
Watanabe M, Ujiie H, Iitani MM, Abe R, **Shimizu H**	Psoriatic onycho-pachydermo periostitis that progressed to generalized pustular psoriasis.	**Clin Exp Dermatol**			in press
Ujiie H, Shibaki A, Nishie W, Shinkuma S, Moriuchi R, Qiao H, **Shimizu H**	Noncollagenous 16A domain of type XVII collagen-reactive CD4(+) T cells play a pivotal role in the development of active disease in experimental bullous pemphigoid model.	**Clin Immunol**			in press
Sasaki K, **Akiyama M**, Yanagi T, Sakai K, Miyamura Y, Satok M, **Shimizu H**	CYP4F22 is highly expressed at the site and onset of keratinization during human skin development.	**J Dermatol Sci**			in press
Natsuga K, Shinkuma S, Kanda M, Suzuki Y, Chosa N, Narita Y, Setoyama M, Nishie W, **Akiyama M**, **Shimizu H**	Possible modifier effects of keratin 17 gene mutation on keratitis-ichthyosis-deafness syndrome.	**Br J Dermatol**			in press
Mizuno O, Yanagi T, Baba K, Yamane N, Inokuma D, Ito K, **Akiyama M**, **Shimizu H**	Sweet's syndrome presenting with vegetative nodules on the hands: relationship to neutrophilic dermatosis of the dorsal hands.	**Int J Dermatol**			in press
Koguchi H, Arita K, Yamane N, Shinkuma S, **Shimizu H**	Erythema annulare centrifugum-like neutrophilic dermatosis:Effects of potassium iodide.	**Acta Derm Venereol**			in press
Izumi K, Yanagi T, **Akiyama M**, Moriuchi R, Arita K, **Shimizu H**	Intractable erythematous plaques on the hands: palmoplantar eosinophilic pustular folliculitis.	**Int J Dermatol**			in press
Homma E, Aoyagi S, Baba K, Iitani M, Hata H, **Shimizu H**	Angiosarcoma on the lower abdominal wall associated with chronic lymphedema in an obese woman.	**Int J Dermatol**			in press
Hirata Y, Abe R, Kikuchi K, Hamasaka A, Shinkuma S, **Nomura T**, Nishie W, Arita K, **Shimizu H**	Intraepidermal neutrophilic IgA pemphigus successfully treated with dapson.	**Eur J Dermatol**			in press

Hayashi I, Shinkuma S, Shimizu S, Natsuga K, Ujiie H, Yasui C, Tsuchiya K, Nishie W, **Shimizu H**	Mucous membrane pemphigoid with generalized blisters: IgA and IgG autoantibodies target both laminin-332 and type XVII collagen.	**Br J Dermatol**			in press
Hamade Y, Arita K, Toyonaga E, Inokuma D, Hamasaka K, **Shimizu H**	Lichen Planus in Childhood Showing Various Cutaneous Features.	**Acta Derm Venereol**			in press
Fujita Y, Inokuma D, Abe R, Sasaki M, Nakamura H, Shimizu T, **Shimizu H**	Conversion from human haematopoietic stem cells to keratinocytes requires keratinocyte secretory factors.	**Clin Exp Dermatol**			in press
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Umemoto H, **Akiyama M**, Yanagi T, Sakai K, Aoyama Y, Oizumi A, Suga Y, Kitagawa Y, **Shimizu H**	New insight into genotype/phenotype correlations in ABCA12 mutations in harlequin ichthyosis.	**J Dermatol Sci**	61	136-138	2011
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Shinkuma S, McMillan JR, **Shimizu H**	Ultrastructure and molecular pathogenesis of epidermolysis bullosa.	**Clin Dermatol**	29	412-419	2011
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Natsuga K, Nishie W, Shinkuma S, Nakamura H, Matsushima Y, Tatsuta A, Komine M, **Shimizu H**	Expression of exon-8-skipped kindlin-1 does not compensate for defects of Kindler syndrome.	**J Dermatol Sci**	61	38-44	2011
Natsuga K, Nishie W, Shinkuma S, Nakamura H, Arita K, Yoneda K, Kusaka T, Yanagihara T, Kosaki R, Sago H, **Akiyama M**, **Shimizu H**	A founder effect of c.1938delC in ITGB4 underlies junctional epidermolysis bullosa and its application for prenatal testing.	**Exp Dermatol**	20	74-76	2011
Nakamura H, Natsuga K, Nishie W, McMillan JR, Sawamura D, **Akiyama M**, **Shimizu H**	DNA-based prenatal diagnosis of plectin-deficient epidermolysis bullosa simplex associated with pyloric atresia.	**Int J Dermatol**	50	439-442	2011
Nakajima K, Sano S, Uchida Y, **Akiyama M**, Morita Y, **Shimizu H**	Altered lipid profiles in the stratum corneum of Sjogren-Larsson syndrome.	**J Dermatol Sci**	63	64-66	2011
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Kikuchi K, Arita K, Tateishi Y, Onozawa M, **Akiyama M**, **Shimizu H**	Recurrence of hydroxyurea-induced leg ulcer after discontinuation of treatment.	**Acta Derm Venereol**	91	373-374	2011
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Kempf M, Miyamura Y, Liu PY, Chen AC, Nakamura H, **Shimizu H**, Tabata Y, Kimble RM, McMillan JR	A denatured collagen microfiber scaffold seeded with human fibroblasts and keratinocytes for skin grafting.	**Biomaterials**	32	4782-4792	2011
Fujita Y, Yoshioka N, Abe R, Murata J, Hoshina D, Mae H, **Shimizu H**	Rapid immunochromatographic test for serum granulysin is useful for the prediction of Stevens-Johnson syndrome and toxic epidermal necrolysis.	**J Am Acad Dermatol**	65	65-68	2011
Fujita Y, Abe R, Nishie W, **Shimizu H**	Regenerative medicine for severe congenital skin disorders: restoration of deficient skin component proteins by stem cell therapy.	**Inflammation and Regeneration**	31	282-289	2011
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Chen AC, McNeilly C, Liu AP, Flaim CJ, Cuttle L, Kendall M, Kimble RM, **Shimizu H**, McMillan JR	Second harmonic generation and multiphoton microscopic detection of collagen without the need for species specific antibodies.	**Burns**	37	1001-1009	2011

Ⅴ．研究成果の刊行物・別刷

British Journal of Dermatology

Original Article

A mutation in the genes encoding γ-secretase underlies familial but not non-familial hidradenitis suppurativa

Running head: A novel *NCSTN* mutation in familial hidradenitis suppurativa

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Abbreviations: HS, hidradenitis suppurativa; APH1A, anterior pharynx defective 1a; APH1B, anterior pharynx defective 1b; APP, amyloid precursor protein; BMI, body mass index; NCSTN, nicastrin; PCR, polymerase chain reaction; PSEN1, presenilin-1; PSEN2, presenilin-2; PSENEN, presenilin enhancer-2; RT-PCR, reverse transcription-PCR; SCC, squamous cell carcinoma

Key words: hidradenitis suppurativa, γ-secretase, nicastrin

Conflicts of interest: None declared.

Bulleted Statements:

What's already known about this topic?

Recently, loss-of-function mutations in the genes encoding γ-secretase were identified as a cause of familial hidradenitis suppurativa (HS) in the Chinese and British populations. However, γ-secretase gene mutations have yet to be identified in other racial populations including Japanese. Furthermore, it remains unclear whether *de novo* mutations within the same genes causing familial HS underlie non-familial HS or not.

What does this study add?

We conducted mutation analysis of the γ-secretase genes in Japanese patients with familial HS. A novel splice site mutation, c.582+1delG, in *NCSTN* that encodes a key component of γ-secretase was identified and confirmed to be a disease-causing mutation using the real-time RT-PCR analysis. We also revealed for the first time that a γ-secretase gene mutation is not linked to the development of non-familial HS.

Summary

Background: Hidradenitis suppurativa (HS) is a chronic follicular occlusive disease with characteristic recurrent draining sinuses, skin abscesses and disfiguring scars, mainly involving the scalp, neck, axilla, groin and perianal and perineal regions. While most HS cases are non-familial, familial cases showing autosomal-dominant inheritance have been reported. Recently, loss-of-function mutations in the genes encoding γ-secretase were identified as a cause of familial HS in the Chinese and British populations.

Objectives: To identify mutations in the genes encoding γ-secretase in Japanese patients with familial and non-familial HS.

Methods: Two affected and three unaffected individuals from a Japanese family with familial HS and nine patients with non-familial HS were recruited. We conducted mutation analysis of the γ-secretase genes in Japanese patients with familial and non-familial HS.

Results: A novel splice site mutation in *Nicastrin,* one of the six key component genes encoding γ-secretase, was identified in the patients with familial HS. Neither unaffected individuals in the family nor 50 unrelated healthy controls carry this mutation. None of the nine patients with

non-familial HS carry nonsense, frameshift and/or splice site mutations.

Conclusions: A novel splice site mutation, c.582+1delG, in *Nicastrin* was identified in the familial HS patients. We also revealed for the first time that a γ-secretase gene mutation is not linked to the development of non-familial HS. These results would further pave the way to a better understanding of the contribution of γ-secretase and other genes to the pathogenesis of HS and to the development of a new therapeutic strategy for HS. (249 words)

Introduction

Hidradenitis suppurativa (HS; also known as Acne inversa; OMIM #142690) is a chronic follicular occlusive disease characterized by recurrent draining sinuses, inflamed nodules and abscesses with subsequent scarring and chronic seepage, mainly involving the intertriginous skin of the scalp, neck, axilla, groin and inframammary, perianal and perineal regions.[1] HS shows female preponderance and usually develops after puberty.[1,2] It has a profound impact not only on the quality of life of the patient, but also the prognosis, since it is associated with an increased risk of cutaneous squamous cell carcinoma (SCC).[3] While most HS cases are non-familial, familial cases showing autosomal-dominant inheritance have been reported.[2,4-9]

Recently, loss-of-function mutations in *Presenilin-1 (PSEN1)*, *Presenilin Enhancer-2 (PSENEN)* and *Nicastrin (NCSTN)*, the genes encoding key components of the γ-secretase protein, were identified as a cause of familial HS in the Chinese population.[2] γ-secretase, an aspartyl protease that cleaves type 1 transmembrane proteins, consists of four essential protein subunits: one catalytic presenilin subunit, encoded by *PSEN1* and *Presenilin-2 (PSEN2),* and three co-factor subunits (presenilin enhancer 2, nicastrin and anterior pharynx defective 1), encoded by *PSENEN, NCSTN,* and *Anterior*

pharynx defective 1A (APH1A) and *Anterior pharynx defective 1B (APH1B),* respectively.[2] Although its overall function remains unclear, the enzyme is known to be required for regulating intramembranous proteolysis of Notch and amyloid precursor protein (APP).[2,10]

Notably, genetic reduction of γ-secretase has been shown to cause SCC and follicular hyperkeratosis via aberrant Notch signaling pathways in mouse models.[11,12] In the development of HS, follicular hyperkeratosis is considered to be the initial event, resulting in follicular occlusion, secondary apocrine involvement and follicular rupture with resultant inflammation and infection. Therefore, loss-of-function mutations in γ-secretase genes, causing reduced γ-secretase activity and decreased Notch signaling, are considered to cause HS. Particularly, missense mutations in *PSEN1* and *PSEN2* have been shown to underlie familial Alzheimer's disease.[13] Although mutations in *PSENEN* and *NCSTN* were subsequently reported in the Chinese and British populations, mutations in the genes encoding γ-secretase have yet to be identified in other racial populations.[6-8]

Here we conducted a mutation analysis of all six genes encoding γ-secretase (γ-secretase genes) in five individuals, including two affected ones, from a Japanese family with familial HS. Furthermore, we carried out direct DNA sequencing of γ-secretase genes in nine unrelated Japanese patients with

non-familial HS, since it remains unclear whether *de novo* mutations within the same genes causing familial HS underlie non-familial HS. To our knowledge, this is the first report of γ-secretase genes analysis in non-familial HS.

Materials and methods

Clinical materials

Blood samples were obtained from two affected and three unaffected members of a Japanese family with familial HS, and from nine Japanese patients with non-familial HS (seven males and two females with a mean age of 38.2 years (range: 15–70 years)). The diagnosis of HS was clinically determined by experienced dermatologists. As mentioned above, a history of five or more painful or discharging nodules, cysts or abscesses in areas frequently affected by HS was required for that diagnosis.[9] For general controls, DNA samples from 50 unrelated ethnically matched individuals were included in the current study. Participants or their legal guardians gave written informed consent in compliance with the Declaration of Helsinki Principles. The study was approved by the Medical Ethics Committee of the

Hokkaido University, Sapporo, Japan.

Genotyping of the γ-secretase genes

We sequenced all exons and exon-intron boundaries of all six γ-secretase component genes (*PSENEN*, *PSEN1, PSEN2, NCSTN*, *APH1A* and *APH1B)* in the Japanese HS patients with or without a family history. Briefly, genomic DNA isolated from peripheral blood was subjected to PCR amplification, followed by direct automated sequencing using ABI PRISM 3130 genetic analyzers (Applied Biosystems, Foster City, CA). The exon-flanking intronic primers used in this study are described in Table 2. 50 control DNA samples (100 alleles) were screened for the mutations c.582+1delG in *NCSTN* and p.Thr421Met in *PSEN2* using direct DNA sequencing.

mRNA expression analysis by real-time RT-PCR

A blood sample from the proband of the family with familial HS was available for RT-PCR analysis. Total RNA was extracted from peripheral lymphocytes using Ficoll-paque PLUS (GE Healthcare, Buckinghamshire,

UK) and then subjected to reverse transcription (RT) with the RNeasy Mini Kit (Qiagen, Hilden, Germany). The cDNA served as a template in quantitative real-time RT-PCR utilizing TaqMan Fast Universal PCR Master Mix (Applied Biosystems) and TaqMan Gene Expression inventoried assay probe (Assay ID; Hs00950933_m1: Applied Biosystems). The assays were performed on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). *NCSTN* mRNA expression was normalized to that of *GAPDH*, and then the relative expression level was determined. The *NCSTN* mRNA expression of the proband was compared with that of the control.

Results

A novel mutation in *NCSTN* underlies familial HS in the Japanese population

We recruited five individuals from a large Japanese pedigree with familial HS (Figure 1a). All were of Japanese origin. The diagnosis of HS was made based on a history of five or more painful or discharging nodules, cysts or abscesses in areas frequently affected by HS, including the axillae, chest, groins, buttocks and/or thighs.[9] Two of the five individuals recruited, the proband (IV-1) and her uncle (III-14), met these criteria (Figure 1a). The proband showed obesity (body mass index (BMI) of 31.2), whereas her uncle's BMI was within normal range. The proband revealed widespread, marked scarring and fistulae, especially on the posterior neck and perineal region (Figures 1b and c). The uncle demonstrated a milder HS phenotype (Figure 1d). The proband's father (III-10) died of SCC on the upper-back that arose in a severely affected HS region.

After DNA was extracted from peripheral blood cells, all exons and exon-intron boundaries of the genes encoding all the six γ-secretase components, *PSEN1*, *PSEN2*, *PSENEN*, *NCSTN*, *APH1A* and *APH1B*, were

amplified by polymerase chain reaction (PCR) and sequenced in five individuals from the family, which led to the identification of a heterozygous single-nucleotide deletion in the *NCSTN* exon 5/intron 5 donor splice site (c.582+1delG) in the two affected individuals (Figure 2a). Notably, the unaffected individuals in the family were all wild-type for the mutation. The mutation was also absent in 100 ethnically matched control alleles. In this family, no one showed any symptoms of Alzheimer's disease or dementia.

Real-time reverse transcription-PCR revealed a remarkable reduction in *NCSTN* mRNA expression in the proband compared with a normal control

To determine whether the splice site mutation in *NCSTN* identified in the proband causes the reduced γ-secretase activity, we extracted mRNA from her peripheral lymphocytes and measured the relative *NCSTN* mRNA level using quantitative real-time reverse transcription-PCR (RT-PCR). It revealed a marked reduction in *NCSTN* mRNA expression in the proband carrying the *NCSTN* splice site mutation compared with a healthy control (Figure 2b),

which suggests that the mutant mRNA was subjected to nonsense-mediated mRNA decay. The real-time RT-PCR experiments were repeated twice, giving almost the same measurement values in each experiment.

Clinical and genetic features of non-familial HS patients

The clinical details of the nine Japanese patients with non-familial HS analyzed in this study are summarized in Table 1. These patients showed typical HS phenotypes, including subcutaneous abscesses, sinuses and scars that mainly affected the scalp, posterior neck, buttocks and/or axillae. Some were refractory to oral antibiotics because of the large draining sinuses and, therefore, had been treated by lesional skin resection and split-thickness skin grafting. Despite the severe symptoms, none of the patients had developed cutaneous SCC on the affected areas. None showed any symptoms or family history of HS or Alzheimer's disease. Mutation analysis of γ-secretase genes was also performed using their DNA samples. Remarkably, no one carried a nonsense, frameshift or splice site mutation in any of the six genes encoding γ-secretase, although patient 4 in Table 1 was heterozygous for the missense mutation, p.Thr421Met in *PSEN2* (Figure 3a).

Discussion

Nicastrin encoded by *NCSTN* is a critical subunit of γ-secretase complex.[14] γ-secretase plays an important role in intramembranous cleavage of Notch and APP.[2,10] In the skin, Notch is expressed in developing or differentiating epidermis and hair follicles, regulating the cell fate.[15] Notably, disruption of a Notch signaling pathway causes epidermal and follicular hyperkeratosis and epidermal cyst formation.[12,15] Therefore, decreased Notch signaling due to loss-of-function mutation in the γ-secretase genes is hypothesized to play a key role in the pathogenesis of HS via aberrant trichilemmal keratinization. This hypothesis is further supported by the fact that HS does not affect the palmoplantar regions, where no hair follicles exist. To date, 11 loss-of-function mutations in *NCSTN*, *PSEN1* and *PSENEN* have been reported in familial HS in the Chinese and British populations, but no loss-of-function mutations have been reported in *PSEN2*, *APH1A* and *APH1B* (Figure 4).[2,6-8] On the other hand, aberrant cleavage of APP is presumed to lead an overproduction of β-amyloid peptides that give rise to the characteristic brain plaques of Alzheimer's disease.[10]

In this study, we identified a novel splice site mutation, c.582+1delG, in *NCSTN*, which was confirmed to be a disease-causing mutation by real-time